Biotechnology and sustainable development

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Introduction

THE PRINCIPLES originally set out in 1987 in the Brundtland Report of the World Commission on Environment and Development, United Nations Environment Program (United Nations, 1987) and reaffirmed at the United Nations Conference on Environment and Development (Rio 92), in the action program of Agenda 21 (United Nations, 1992) and, more recently, in the Millennium Development Goals established in 2000 (United Nations, 2000), identified as a priority for the future of humanity, the adoption of a new, so-called sustainable development paradigm, to ensure progress while preserving the environment. Achieving the sustainable development goals implies necessarily the rational management of natural resources, which will require the use of new technologies. Among the technologies that have the potential to contribute to sustainable development, biotechnology has much to offer, especially in the fields of food production, energy generation, prevention of environmental pollution and bioremediation.

The present article describes some of the possible biotechnological routes relevant to sustainable development, with examples of studies carried out in our research group.

Energy generation: increased productivity of ethanol through genetic improvement of yeast

Currently, fossil fuels (coal, oil, natural gas) account for approximately 80 percent of global primary energy needs. On the other hand, the world energy demand is expected to increase 49 percent by 2035, while the production of oil - a non-renewable product - still tends to increase over the next 25-30 years (Energy 2010).

Even if future consumption of fossil fuels is limited to today's proven reserves, the burning of these fuels would result in the release of more than twice the total amount of carbon that is currently in the atmosphere, thereby exacerbating the greenhouse effect. In fact, the use of fossil fuels is a major cause of greenhouse gas emissions, which are mainly responsible for the climate changes we have been experiencing. Thus, replacing gasoline with biofuels, such as ethanol, is a biotechnological solution to avoid future problems of energy shortage and severe environmental changes (Um futuro ..., 2010).

Ethanol production by fermentation is, by definition, a biotechnological process, as the agent responsible for transforming sugar into alcohol - the yeast *Saccharomyces cerevisiae* is a living organism. This yeast has been used by man for at least eight thousand years for the production of food and beverages, among other products of considerable economic relevance.

To date, S. *cerevisiae* remains the most widely used microorganism for the industrial production of ethanol, because of its high selectivity in ethanol production, high growth and fermentation rate, high ethanol yield, high tolerance to glucose, ethanol, osmotic pressure and stressful conditions, low optimum fermentation pH and high optimum fermentation temperature (Amorim & Leão, 2005).

Among the different factors that influence the performance of yeast in the ethanol production process is the bacterial contamination of the fermentation medium, which is responsible for significant losses in the productivity of distilleries. In addition to competing with yeast for sugar and other broth nutrients, bacteria introduce undesirable products of their metabolism, which cause adverse effects to the yeast fermentation process (Andrietta et al., 2007). The measures currently used to control these contaminants involve acidification of the broth and the use of antibiotics. For acidification, sulfuric acid is added to the preparation of the broth, considerably reducing bacterial contamination. However, the repeated use of acidification also reduces yeast viability.

Another widespread practice in alcohol plants involves adding antibiotics to the broth, which, however, significantly increases the cost of the process, besides negatively impacting the environment. The continuous use of antibiotics also entails the selection of resistant bacteria and the consequent outgrow of a new population of contaminants, thus requiring the use of new antibiotics or even of mixtures of various antibiotics (Amorim & Leão, 2005).

Aiming to find an alternative solution to combat bacterial contamination in the industrial process of ethanol production, a new line of research was introduced in our laboratory several years ago, to develop a *Saccharomyces* yeast strain able to produce and excrete to the fermentation medium a bactericidal substance, while retaining its high fermentative abilities.

Among the proteins with bactericidal power lysozyme is noteworthy, since there are no reports about the development of resistance mechanisms among species of bacteria isolated from the fermentation process. Thus, if the yeast itself could excrete lysozyme to the fermentation medium, a new, simpler, less costly and more environmental-friendly way to combat bacterial contamination in the industrial process would be achieved.

Lysozyme (EC 3.2.1.17) is an enzyme that specifically catalyzes the hydrolysis of the β-(1.4)-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan, which is the main constituent of the bacterial cell wall. It is an enzyme widely distributed among living organisms

(but absent in yeast), being extremely effective in the defense against bacterial infections (Kirby, 2001). This enzyme is particularly effective against gram-positive bacteria, which are common in Brazil distilleries, representing 98.5 percent of the contaminants (Gallo, 1989). In turn, lysozyme D, produced by *Drosophila melanogaster* (the fruit-fly), has characteristics that enable its full operation during the fermentation process, namely optimal enzymatic activity in acidic environment (its optimum pH is 3.5), and resistance to proteolysis and heat (Kylsten et al., 1992).

Based on this information, an expression-secretion cassette of the cDNA of *D. melanogaster* lysozyme D was constructed in our laboratory, under control of the *S. cerevisiae* alcohol dehydrogenase I gene promoter (Grael, 1998, 2010). Next, this expression-secretion cassette was integrated into the chromosome V of *S. cerevisiae*, which ensured full stability of the cloned information in the yeast strain, without loss of productivity. This methodology enabled the transformation of laboratory strains as well as of strains used in the industry. The transformant strains produce active lysozyme, which is secreted into the medium from the beginning of fermentation, and are capable of hydrolyzing the wall of *Micrococcus lysodeikticus* and inhibiting the growth of *Bacillus coagulans* and *Lactobacillus fermentum*, the main bacterial contaminants of alcoholic fermentation (Gallo, 1989). Furthermore, the yeast transformants show 100 percent stability, which is a highly relevant aspect for the industrial process (Silveira, 2003; Silveira et al., 2003).

An additional advantage of the system we have developed is the fact that the lysozyme produced during fermentation can be purified from the supernatant for use in food preservation and in the composition of pharmaceutical drugs (Proctor and Cunningham, 1988), thereby adding value to the ethanol production process.

This line of research gave rise to two patent applications, one of which has already been granted (Grael, 2010).

Prevention of environmental pollution: production of biopolymers from renewable resources

To solve the issue of urban and industrial waste, the replacement of plastics of petrochemical origin with plastic produced by microorganisms would be highly desirable, since biopolymers are bio-compatible and fully biodegradable materials. The estimate is that plastic waste dumped in landfills increases by 404 percent the total weight of the waste and the problem is worsened by the fact that the plastic materials currently produced are of difficult decomposition, remaining in the environment for several hundred years. However, the price of biopolymers is not yet able to compete with conventional plastics, making it necessary to optimize the microbial strains, as well as the extraction and recovery processes, but especially to reduce the costs of raw materials (Choi & Lee, 1999). It is possible, however, to foresee that the current situation will change

as oil becomes scarce, making products made from renewable raw materials less expensive than products from the petrochemical industry.

Construction of new bacteria for the production of biopolymers from sucrose

Polyhydroxyalkanoates (PHAs) are polymers produced by many bacteria as storage material in the form of granules, when there is an abundance of carbon source. PHAs have thermoplastic properties comparable to those of plastics of petrochemical origin, in addition to being fully biodegradable (Madison & Huisman, 1999). This property makes PHAs very important with regard to environmental protection. In addition, the use of PHAs would contribute to sustainable development, since they are produced by microorganisms from natural renewable resources. However, the economically feasible use of PHAs as substitutes for plastics of petrochemical origin requires abundant low-cost raw materials, on which PHA-producing bacteria can be cultured. In Brazil, sugarcane meets these requirements to be used as raw material. However, the bacterium *Cupriavidus necator* (formerly *Alcaligenes eutrophus*, *Ralstonia eutropha*, *Wautersia eutropha*), which is an excellent PHA producer (Reinecke & Steinbüchel, 2009), is incapable of using the sucrose contained in sugarcane juice. The study conducted in our research group involved the genetic improvement of C. *necator* DSMZ 545, aiming to enable it to grow on sucrose as the sole carbon source. To achieve this goal, a five-gene operon from the *Salmonella typhimurium* plasmid pUR400, which encodes all the enzymes necessary for sucrose assimilation, was added to the genome of this strain (Fava, 1997; Vicente et al., 2009). This genetically modified *C. necator* strain was then mutagenized to increase its efficiency in converting the propionate precursor into hydroxyvalerate units in the polyhydroxybutyrate-polyhydroxyvalerate copolymer (Sartori, 1998, Vicente et al., 1998). In fact, this copolymer is of great technological relevance because it shows greater flexibility than the homopolymer, thus increasing its use range in the plastics industry (Madison & Huisman, 1999). This study gave rise to two patent applications with the National Institute of Industrial Property (INPI) (Vicente et al., 2009; Vicente, 1998), which have already been granted for industrial use.

Bioremediation of water contaminated with toxic metals

Bioremediation is the use of living organisms to decontaminate or reduce the content of pollutants in the environment. Indeed, several plants and microorganisms are able to accumulate and transform different pollutants into less toxic substances (Atlas & Unterman, 1999). Given that conventional environmental remediation technologies are generally inadequate to reduce to acceptable levels the concentrations of heavy metals in contaminated effluents, bioremediation has become an alternative solution of great interest. Furthermore, biotechnological methods for the detoxification of effluents are less expensive than conventional technologies. In fact, in the case of surface water contami-

nation with heavy metals, *in situ* immobilization of metal ions by microbial action, which prevents them from being transferred to the water table, is an economically interesting solution (Gravilescu, 2004). In recent years, the action of microorganisms on metals has been the subject of numerous studies because of their potential use for both detoxification and recovery of metals in mining activities (bioleaching).

Beyond a certain level of concentration, heavy metals are extremely toxic to living organisms, which consequently have developed different biological defense mechanisms throughout the evolution process. Among these mechanisms, some may be useful in bioremediation processes. There are microorganisms that secrete substances that cause the precipitation of metals in an insoluble form (biomineralization); others that internalize metal ions by active transport processes (bioaccumulation); and others yet that passively adsorb metal ions on the cell surface (biosorption) (Barkay & Schaefer, 2001). These different processes are valid for the decontamination of water polluted with heavy metals, although today biosorption is the most widely used approach. Biosorption is particularly effective for the treatment of wastewater, with a more limited use for the bioremediation of soils (ibid.). In addition, the adsorbed metals can be recovered by acid treatment with chelating agents or even by incineration of microorganisms. In fact, bacteria, fungi and yeasts, which are residues from industrial fermentations, can in principle serve as an inexpensive material for the bioremediation of metal-contaminated water.

There are also several plants that concentrate heavy metals in important proportions of their dry weight, such as *Brassica juncea*, which is able to concentrate more than 40 percent. Phytoremediation is therefore an interesting alternative for cleaning water contaminated with metals and radionuclides, and this strategy was used to decontaminate water at Chernobyl, Russia, following the nuclear accident. However, hyperaccumulator plants present slow growth, and the genetic control of accumulation mechanisms is not yet sufficiently clear (Lasat, 2002). Since the mechanisms of plant resistance to heavy metals are different from those of bacteria, it is possible, through genetic engineering techniques, to use plant genes to construct novel bacterial strains with higher bioremediation capacities. Indeed, the cloning in *Escherichia coli* of a gene of the plant *Arabidopsis thaliana* involved in resistance to metals, yielded very promising results to improve the ability of the bacteria to accumulate cadmium, copper and arsenic (Sauge-Merle et al., 2003).

To respond to the presence of high concentrations of heavy metals that are toxic to them, eukaryotic organisms produce cysteine-rich peptides such as glutathione (Singhal et al., 1997), metallothioneins (Mt) (Stillman et al., 1992) and phytochelatins (PC) (Rauser, 1995). These molecules bind to and sequester metal ions in a biologically inactive form.

Phytochelatins are short peptides, whose general structure is (γGlu-Cys)

n Gly (n=2 - 11) (ibid.). PCs have advantages over MTs due to their structural characteristics, particularly the repetition of γ-Glu-Cys units. PCs are more stable and have higher metal-binding capacity than MT. Moreover, PCs can incorporate high levels of inorganic sulfide, which results in a strong increase in their capacity to bind to Cd^{2+} ions (Bae et al., 2000). However, PC production by genetic engineering is not yet possible due to lack of sufficient knowledge about the enzymes involved in the synthesis and elongation of these peptides. PC analogues have been synthesized which, instead of the γ bond, present an α-peptide bond between Glu and Cys, in such a way that these analogs (ECs) can be produced by the ribosomal machinery of the cell. Furthermore, ECs of different chain lengths (longer than those found in plants) can be produced, with different metal-binding capacities (Bae & Mehra, 1997).

Bae et al. (2000) cloned in *E. coli* synthetic genes encoding ECs. These authors constructed fusions between ECs of different lengths and Lpp-OmpA, an *E. coli* surface protein, and found that a chain of only 20 polymeric units of ECs has a binding capacity to Cd^{2+} ions 40 percent higher than mammalian metallothioneins.

Another interesting approach is to genetically improve certain microorganisms that have a high natural tolerance to heavy metals. This is the case of the *Cupriavidus metallidurans* bacterium, which is able to grow in the presence of millimolar concentrations of toxic metals.

C. metallidurans is found in water and soils with a high content of heavy metals and has multiple resistances (Zn, Cd, Co, Pb, Cu, Hg, Ni, and Cr), thanks to two megaplasmids containing the genes involved in a very efficient cation efflux mechanism (Von Rozycki & Nies, 2009). This mechanism of resistance detoxifies the cytoplasm of the bacterium, but does not lend itself to be used in bioremediation. However, it would be possible to take advantage of this bacterium's high resistance to metals and provide it with the genes necessary for the immobilization of metal ions. In fact, a three-fold increase was described in the binding capacity to cadmium in *R. metallidurans*, by cloning the gene encoding the mouse metallothionein I, so as to target this MT to the surface of the bacterium (Valls et al., 2000). These authors have also showed that the inoculation of this genetically modified strain of *R. metallidurans* in soils contaminated with Cd^{2+} ions significantly decreases the toxic effects of cadmium on the growth of tobacco plants (ibid.).

Given the ubiquity of microorganisms capable of immobilizing metals in nature and considering that their frequency is generally increased in contaminated soil and water, stimulation of the microflora indigenous to the contaminated sites is an approach that can also produce good results. In turn, the use of microorganisms with special metabolic activities capable of supplementing the indigenous microflora should also be considered. In any case, genetic engineering techniques can be used to increase the efficiency of microorganisms

for bioremediation, although biosafety measures are required for the release of recombinant organisms for *in situ* bioremediation processes (Urgun-Demirtas et al., 2006).

A project is under way in our laboratory for the construction of various recombinant microorganisms (bacteria and yeast), which have increased capacity to accumulate heavy metals. To achieve this goal, we have chosen the strategy of adding to the genome of different strains, the gene encoding a synthetic phytochelatin using specific expression-secretion cassettes. Aiming for maximum environmental protection, a strongly regulated gene, whose expression will promote its death after having played the role of bioremediation, will also be incorporated to these genetically improved strains. On the other hand, strains are being constructed to be used as biosensors of various metal ions.

One of the subprojects, which has already been completed, aimed to enable the bacterium *Cupriavidus metallidurans* CH34 to adsorb metal ions on its surface. The *C. metallidurans* CH34 (formerly *Alcaligenes eutrophus*, *Ralstonia eutropha*, *Ralstonia metallidurans* and *Wautersia metallidurans*) is the most resistant organism to heavy metal ions known to date. It is a gram-negative, non-pathogenic β-proteobacterium, capable of growing in high concentrations of at least thirteen different heavy metal ions (Von Rozycki & Nies, 2009). This strain was isolated in sediments of zinc settling ponds in Liège, Belgium (Mergeay et al., 1978), and is highly resistant to Ag^{2+}, Bi^{3+}, Cd^{2+}, Co^{2+}, $CrO4^{2-}$ Cu^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, $SeO4^{3-}$, Tl^{1+} and Zn^{2+} conferred by at least 150 resistance genes present in its four replicons: the chromosome (3.9 Mb), a megaplasmid (2.6 Mb), and two plasmids, pMOL30 (234 Kb) and pMOL28 (171Kb) (Von Rozycki & Nies, 2009).

The main resistance mechanism of *C. metallidurans* CH34 is, however, a cation efflux system, which effectively detoxifies the cytoplasm of the bacterium, but not the environment and, therefore, this bacterium is not suitable for bioremediation. For this bacterium to fully develop its biotechnological potential, it would need to be subject to genetic manipulations that could give it the ability to immobilize heavy metal ions on its cell surface, making the strain capable of serving as a bioremediation agent, in addition to colonizing environments containing metals.

By using a strong metal-induced promoter developed in our laboratory (Ribeiro-dos-Santos et al., 2010), we have constructed a new strain of C. *metallidurans* CH34 capable of removing seven different metal ions (Pb^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+}) from the medium in which it is placed, at levels significantly higher than the ones presented by the wild-type strain, as shown in the results presented in Table 1.

Table 1 - Metal biosorption using the recombinant bacterium C. metallidurans CH34 carrying the pCM2 plasmid. The tests were performed with the recombinant bacterium before and after inducing the synthesis of the EC20 phytochelatin. The wild bacterium was used as negative control (high-resolution mass spectrometry with inductively activated plasma source - ICP-MS). The percentage indicates the increase in adsorption relative to the wild-type strain.

	µM Cd^{2+} mg dw dw	µM Co^{2+} mg dw os	µM Cu^{2+} mg dw dw	µM Hg^{2+} mg dw dw	µM Mn^{2+} mg dw dw	µM Ni^{2+} mg dw dw dw	µM Zn^{2+} mg dw dw	µM Pb^{2+} mg dw dw
C. metallidurans CH34	11.1		24.29	0.05	4.73	5.84	16.00	17.62
C. metallidurans CH34/ pCM2	12.6	5.0	25.72	0.05	5.06	6.46	18.82	25.14
C. metallidurans CH34/ pCM2 induced	17.6 (40%)	5.1 (13%)	42.65 (76%)	0.05 -	6.2 (31%)	8.46 (45%)	51.07 (219%)	54.75 (210%)

To achieve this goal, we added to the genome of the original strain the gene sequences necessary for the bacterium to express, anchored on its surface, a synthetic protein with high metal-binding capacity (phytochelatin EC20). Actually, the new bacterium acts as a magnet for metals, becoming completely covered by metal ions and remains perfectly healthy during the process. Thus, while it may be used for the decontamination of any metal-containing effluent (bioremediation), this bacterium can be used to concentrate and recover the metals lost during the ore extraction process (bioleaching). The advantages of this type of treatment are its low cost and high efficiency compared to the physical-chemical methods currently used. The new bacterium is particularly interesting for the mining industry, as it is able to increase the productivity of ore extraction while reducing the environmental impacts of these activities. This project was funded by Cia. Vale, with the aim of using the bacterium in bioreactor for the treatment of mining effluents. This study gave rise to two patent deposits (Schenberg et al., 2008; Biondo et al., 2008).

References

AMORIM, H. V.; LEÃO, R. M. *Fermentação alcoólica*: Ciência e Tecnologia. Piracicaba: Fermentec, 2005.

ANDRIETTA, M. G. S. et al. Bioethanol – 30 years of Proalcool. *International Sugar Journal*, v.109, p.195-200, 2007.

ATLAS, R. M.; UNTERMAN R. Bioremediation. In: DEMAIN, A. L.; DAVIES, J. E. (Ed.) *Industrial microbiology and biotechnology*. s. l.: ASM Press, 1999. p.666-81.

BAE, W.; MEHRA, R. K. Metal-binding characteristics of a phytochelatin analog. *J. Inorg. Biochem.*, v.68, p.201-10, 1997.

BAE, W. et al. Enhanced bioaccumulation of heavy metals by bacterial cells displaying synthetic phytochelatins. *Biotechnol. Bioeng.*, v.70, p.518-24, 2000.

BARKAY, T.; SCHAEFER, J. Metal and radionuclide bioremediation: issues, considerations and potentials. *Curr. Opin. Microbiol.*, v.4, p.318-23, 2001.

BIONDO, R. et al. Plasmídeo recombinante contendo cassete de ancoragem, linhagem bacteriana contendo o plasmídeo recombinante e seu uso. BR PI 0801282-2, 1º abr. 2008.

CHOI, J.; LEE, S. Y. Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation. *Applied Microbiol. Biotechnol.*, v.51, p.13-21, 1999.

ENERGY Information Administration/International Energy Outlook 2010.

FAVA , A. L. B. *Clonagem e expressão do regulon scr em* Alcaligenes eutrophus *visando a produção de polihidroxibutirato a partir de sacarose*. São Paulo, 1997. Dissertation (MSc) – Microbiology Program, University of São Paulo.

GALLO, C. R. *Determinação da microbiota bacteriana de mosto e de dornas de fermentação alcoólica*. Campinas, 1989. Thesis (PhD) – School of Food Engineering, State University of Campinas.

GAVRILESCU, M. Removal of heavy metals from environment by biosorption. *Eng. Life Sci.*, v.4, p.219-32, 2004.

GRAEL, E. T. *Clonagem e expressão do cDNA de lisozima de* Drosophila melanogaster *em* Saccharomyces cerevisiae *visando o controle de contaminantes bacterianos da fermentação alcoólica*. São Paulo, 1998. Thesis (PhD) – Microbiology Program, University of São Paulo.

GRAEL, E. T. et al. Cepa transgênica de *Saccharomyces* sp que produz e secreta lisozima ácida, método de obtenção e usos da cepa transgênica. BR PI 9806386-3 B1, 01/12/1998. Patent granted 29 June 2010.

KIRBY, A. J. The lysozyme mechanism sorted – after 50 years. *Nature Structural Biol.*, v.8, p.737-9, 2001.

KYLSTEN, P. et al. The lysozyme locus in *Drosophila melanogaster*: different genes are expressed in midgut and salivary glands. *Mol. Gen Genet.*, v.232, p.335-43, 1992.

LASAT, M. M. Phytoextraction of toxic metals. *J. Environ. Qual.*, v.31, p.109-20, 2002.

MADISON, L. M.; HUISMAN, G. W. Metabolic engineering of poly (3-hydroxyalcanoates): from DNA to plastic. *Microbiol. Mol. Biol. Rev.*, v.63, p.21-53, 1999.

MERGEAY, M. et al. Extrachromosomal inheritance controlling resistance to cadmium, cobalt, copper, and zinc ions: evidence from curing in a *Pseudomonas*. *Arch. Int. Physiol. Biochim.*, v.86, p.440-2, 1978.

PROCTOR, V. A.; CUNNINGHAM, F. E. The chemistry of lysozyme and its use as a food preservative and a pharmaceutical. *Crit. Rev. Food Sci. Nutr.*, v.26, p.359-95, 1988.

RAUSER, W. E. Phytochelatins and related peptides. *Plant. Physiol.*, v.109, p.1141-9, 1995.

REINECKE, F.; STEINBÜCHEL, A. *Ralstonia eutropha* strain H16 as model organism for PHA metabolism and for biotechnological production of technically interesting biopolymers. *J. Mol. Microbiol. Biotechnol*, v.16, p.91-108, 2009.

RIBEIRO-DOS-SANTOS, G. et al. A metal-repressed promoter from Gram-positive *Bacillus subtilis* is highly active and metal-induced in Gram-negative *Cupriavidus metallidurans*. *Biotechnol. Bioeng*, v.107, p.469-77, 2010.

SARTORI, D. M. *Obtenção de um mutante de* Alcaligenes eutrophus *melhorado geneticamente para a produção do co-polímero polihidroxibutirato-polihidroxivalerato (PHBPHV*). São Paulo, 1998. Dissertation (MSc) – Microbiology Program, University of São Paulo.

SAUGE-MERLE, S. et al. Enhanced toxic metal accumulation in engineered bacterial cells expressing *Arabidopsis thaliana* phytochelatin synthase. *Appl. Environ. Microbiol.*,v.69, p.490-4, 2003.

SCHENBERG, A. C. G. et al. Promotor heterólogo para expressão de proteínas em bactérias e seu uso em aplicações biotecnológicas. BR PI 0801277-6, 1st Apr. 2008.

SILVEIRA, L. J. *Construção de uma linhagem de levedura auto-suficiente no combate às contaminações bacterianas da fermentação alcoólica*. São Paulo, 2003. Dissertation (MSc) – Biotechnology Program, University of São Paulo.

SILVEIRA, L. J. et al. Plasmídeo recombinante, método de obtenção de uma linhagem de levedura transgênica e linhagem de levedura transgênica. BR PI0306946-0, 14 Nov. 2003.

SINGHAL, R. K. et al. Glutathione, a first line defense against cadmium toxicity. *Faseb J.,* v.1, p.220-3, 1997.

STILLMAN, N. J. et al. *Metallothioneins*. Berlin: VCH Publishers, 1992.

UM FUTURO COM ENERGIA SUSTENTÁVEL: iluminando o caminho. São Paulo: Research Support Foundation of the State of São Paulo (FAPESP); Amsterdam: Inter Academy Council; Rio de Janeiro: Academia Brasileira de Ciências, 2010.

UNITED NATIONS. Report of the World Commission on Environment and Development, United Nations General Assembly, 96th plenary meeting, 11 December 1987, Document A/RES/42/187. Available at: <www.un.org/documents/ga/res/42/ares42-187.htm>.

URGUN-DEMIRTAS, M. et al. Use of genetically engineered microorganisms (GEMs) for the bioremediation of contaminants. Crit. Rev. Biotechnol., v.26, p.145-64, 2006.

VALLS, M. et al. Engineering a mouse metallothionein on the cell surface of *Ralstonia eutropha* CH34 for immobilization of heavy metals in soil. *Nat. Biotechnol.*, v.18, p.661-5, 2000.

VICENTE, E. J. et al. Cepa mutante de *Alcaligenes eutrophus*, cepa transgênica de mutante de *Alcaligenes eutrophus* e método de obtenção. BR PI 9805116-4, 14 Aug. 1998.

VICENTE, E. J. et al. Cepa transgênica de *Alcaligenes eutrophus* e método de obtenção de cepa transgênica de *Alcaligenes eutrophus*. BR PI 9806581-5 A, 12/08/1998. Patent granted 15 Dec. 2009.

VON ROZYCKI, T.; NIES, D. H. *Cupriavidus metallidurans*: evolution of a metal resistant bacterium. *Antonie van leeuwenhoek*, v.96, p.115-39, 2009.

Abstract – Biotechnology can play an important role to reach the goals of sustainability. In the present work, we describe successful examples of microorganisms especially designed for optimizing ethanol production, biodegradable plastics production from renewable resources, and toxic metals bioremediation. These biotechnological processes significantly contribute to promote sustainable development, although they may, at present, not be competitive with the conventional technologies.

Keywords: Biotechnology, Sustainable development, Biofuels, Biopolymers, Bioremediation.

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